

TWO NEW SPECIES OF
MONOBLEPHARELLA

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TWO NEW SPECIES OF MONOBLEPHARELLA¹

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(WITH 51 FIGURES)

Soils collected from localities widely distributed in the Western Hemisphere have yielded a number of isolates of fungi referable to the genus *Monoblepharella*. A study has been made of the development and morphology of the ten isolates producing sex organs, and will be reported in a later paper. Cultures of the original isolates of *Monoblepharella Taylora* Sparrow and *M. mexicana* Shanor were furnished by the authors. It was possible, therefore, to make careful comparisons with the only two members of the genus which have already been described. This study has resulted in the addition to the genus of two new species.

***Monoblepharella elongata* sp. nov.**

Mycelium well developed; hyphae sparingly branched, the branches usually arising at right angles to the main axes, 1.5-4 μ in diameter, the stouter basal portions up to 7 μ ; vacuolization reticulate or scalariform; hyphae usually with many irregular swellings. Sporangia narrowly cylindrical or siliquiform, 40-120 μ in length by 5-13 μ in diameter at the widest point, tapering to 2-4 μ at the base, terminal, or after sympodial branching of the hyphae, appearing lateral, usually with one or occasionally several lateral papillate outgrowths near the base. Zoospores emerging through a pore at the apex of the sporangium and through the apices of outgrowths; zoospore with an anterior group of small refractive globules, ovoid or subcylindrical, 6.5-10.5 μ long by 4-6 μ wide, the single posterior flagellum up to 26 μ long. Oogonium at first terminal or, after sympodial branching of the supporting hypha, appearing latera!

¹ Contribution from the Botany Department, University of Michigan, no. 744.

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narrowly obpyriform, 17–35 μ long by 7–12 μ in diameter at the widest point, with rounded apex and a cylindrical base 2–4 μ in diameter, the contents at maturity forming one to three or rarely up to eight eggs containing numerous large refractive globules. Antheridium either terminal on a branch subtending the oogonium and cylindrical, 20–45 μ long by 3–5 μ wide, or hypogynous and geniculate, consisting of a cylindrical section of the suboogonial hypha 2–19 μ long and a beaklike lateral outgrowth 10–35 μ long by 3–5 μ wide. Antherozoids four to seven, emerging through a pore at the apex of the antheridium, strongly amoeboid, ovoid when swimming, 5–6 μ long by 3–4 μ wide, with an anterior group of small refractive globules, posteriorly uniflagellate, the flagellum up to 20 μ in length. Zygote spherical or broadly ovoid, 9–13 μ long by 8–10 μ wide, posteriorly uniflagellate, free swimming or during rest periods strongly amoeboid, containing large refractive globules. Oospore formed free in the water, or occasionally retained within or at the orifice of the oogonium, 9–12 μ in diameter, with a light brown, smooth wall up to 1 μ in thickness, contents bearing globules, upon germination forming a mycelium.

In soil, isolated on hemp seed bait. Soil collected by C. D. LaRue from ditch behind rubber factory, Las Palmas, State of Chiapas, Mexico, February 6, 1941 (type of species). Soil collected by M. E. Springer from marble quarry, Columbia, Tuolumne County, California, March 28, 1942.

Mycelium amplum, hyphis ramosis, tenuibus 1.5–7 μ diametro, reticulate vel scalariforme vacuolatis, plerumque multis irregularibus tumoribus. Sporangia anguste cylindrica vel siliquiformia, 40–120 μ longa, 5–13 μ crassa, basi angustata 2–4 μ crassa, plerumque uno aut rare compluribus papilliformis protrusionibus ad basum; zoosporis ovoideis vel subcylindricis, 6.5–10.5 μ longis, 4–6 μ crassis, uno postico flagello usque ad 26 μ longo. Oogonium hypham terminans, anguste obpyriforme, 17–35 μ longum, 2–4 μ crassum, apice rotundato, basi anguste cylindrico 2–4 μ diametro, ovis 1–3 rare ad 8 cum globulis magnis refractivis. Antheridium aut hypham oogonio subnatam terminans, anguste cylindricum, 20–45 μ longum, 3–5 μ diametro, aut hypogynum, segmenti hyphae suboogonialis 2–19 μ longi et protrusionis rostriformae lateralis 10–35 μ longi, 3–5 μ crassi; antherozoides 4–7 valde amoeboides, ovoideis si natantibus, 5–6 μ longis, 3–4 μ crassis, uno postico flagello ad 20 μ longo. Ova inseminata sphaerica vel late ovoidea, 9–13 μ longa, 8–10 μ crassa, uno postico flagello, natantia aut, dum requiescent, valde amoeboides, globulis magnis refractivis inclusis. Oospora in aqua libere formata sphaerica, 9–12 μ diametro, membrana laevia pallide brunnea, ad 1 μ crassa, globulis inclusis, germinata mycelium formans.

In humo per *Cannabis* semen illecebram culta. Humum legit C. D. LaRue e fossa, Las Palmas, Civitas Chiapas, Mexico, Feb. 6, 1944 (specimen typicum).

Monoblepharella elongata appears to be most closely related to *M. Taylora* Sparrow. The order of development and the arrangement of the organs is the same. The most conspicuous difference between the two species is that which suggested the specific name *elongata* for the new species—a pronounced elongation in *M. elongata* of all of the organs, sporangia, as well as antheridia and oogonia, without a corresponding increase in diameter.

Many of the stages in the morphology and development of *Monoblepharella elongata* are similar to those which have already been described for *Monoblepharis* (Sparrow, 1933, 1943) and for other species of *Monoblepharella* (Sparrow, 1940; Shanor, 1942). The mycelium, like that of other species of *Monoblepharella*, is sufficiently distinctive to identify it as belonging to the genus even in the vegetative condition. It grows very slowly and is so delicate that it is distinguishable even from other members of the *Monoblepharidales*, all of which possess the same type of reticulate or scalariform vacuolization. Globose or irregular swellings (FIG. 9) occur in great abundance on the hyphae of *M. elongata*. Such nodules are found to some extent on the hyphae of all other species (Sparrow, 1940; Shanor, 1942), but not in such large numbers. They are considered to be normal structures, probably acting as reservoirs of protoplasm for subsequent vegetative growth.

In undisturbed cultures only a few reproductive organs will develop at any one time. Although both nonsexual and sexual organs are occasionally found simultaneously, more typically either the one or the other is formed under given conditions. It has been found that production of sporangia is stimulated by the sudden starving of the mycelium by placing it in fresh water at a temperature preferably between 21 and 31° C. Sex organs are formed in abundance only when the mycelium is left undisturbed for several days at constant temperatures ranging from 26–32° C., with the optimum in this species from 26–27° C.

By reason of their refractive appearance and large size as compared with the delicate mycelium, the sporangia of *Monoblepharella elongata* are very conspicuous. Although there is much overlap in the measurements of the sporangia of this and of other species, the average length in *M. elongata*, 75 μ , is much greater while the average width, 8.6 μ , is somewhat less. The mean ratio

of length to diameter is more than one and one-half times that of *M. Taylori*. The presence of lateral papilla-like projections has been mentioned for *M. Taylori* and *M. mexicana*, but in both of these species they occur only rarely. The sporangia of *M. elongata* (FIGS. 1, 2, 4) typically bear one or more of these lateral projections which are used as additional exit tubes, although the majority of the zoospores escape through the apex. The zoospores in the manner of their escape and appearance (FIG. 3) are indistinguishable from those described for other species.

Both oogonia and antheridia are at once distinguished from those of congeneric forms by their long narrow shape and the frequency with which more than one egg is formed in the oogonium. The method of development and the resulting arrangement of sex organs are like those described in *Monoblepharella Taylori* (Sparrow, 1940). The oogonial rudiment develops terminally on a hypha (FIG. 5). Small oil droplets coalesce to form the large globules (FIG. 6) characteristic of the mature egg. Although at first they fill the oogonium, later the globules are found only in the central portion, leaving an area of clear protoplasm at the tip and at the base. At maturity the egg rounds up and withdraws from the base of the oogonium. If more than one egg is to be formed in the oogonium, this is indicated by a characteristic grouping of the oil globules before the actual delimitation is noticeable. In about one-half of the oogonia more than one egg is produced. The number is usually from one to three, although eight were observed in a single oogonium (FIG. 12). This is in striking contrast to the other species where it is only rarely that an oogonium contains more than one egg.

Antheridial growth is initiated by the development of a short lateral branch arising immediately beneath the oogonial cross wall (FIG. 6). The antheridium may be terminal (FIGS. 7, 11) or hypogynous (FIGS. 8-11), with both types sometimes found even on the same branch (FIG. 11). The antheridia average about eight times as long as wide, while in *M. Taylori* this ratio is only about three to one. The emergence of the antherozoids (FIG. 13) is similar to that of the zoospores. While emerging the antherozoid is round or irregular at first, but when swimming it resembles a small zoospore (FIG. 14). When it is moving about on the surface



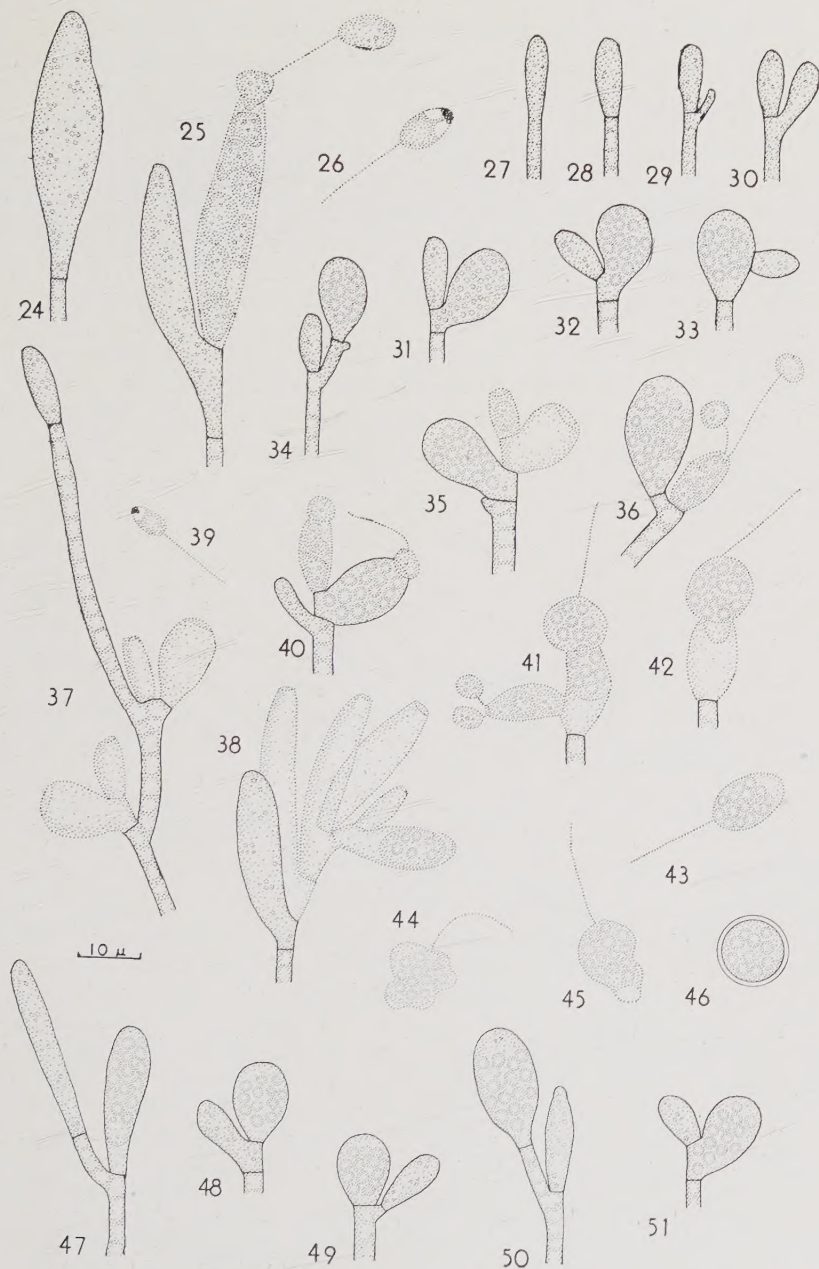
FIGS. 1-23. *Monoblepharella elongata*.

of an oogonium (FIG. 15), it is strongly amoeboid, its flagellum remaining passive, or waving weakly about in the medium. Fertilization and the emergence of the zygote (FIGS. 16-19) occur precisely as in *M. Taylora*. Indeed, save for the difference in the shape of the sex organs, the figures of this process as occurring in *M. Taylora* (Sparrow, 1940) could be used for any of the species studied. The zygote (FIG. 20) too is characteristic of the genus. As it swims with a rolling, halting and rocking movement, it progresses so slowly that it is frequently possible to see the moving posterior flagellum. The flagellum may be visible throughout the entire period of fertilization, emergence, and the early swimming stages, thus giving definite evidence that it is, as Sparrow contended, the flagellum of the antherozoid which is used for the propulsion of the zygote. As the zygote rolls and turns the globules are easily distinguishable and seem to form a compact peripheral layer lining all except the broadly conical anterior end. Motility may continue for a period of at least half an hour, during which time it frequently stops to rest or to undergo strong amoeboid crawling (FIG. 21). Finally it encysts and becomes surrounded by a smooth thickened wall (FIG. 22).

Germination of the oospores may be obtained by adding fresh water and bait to an old culture or to the dried spores. It is not known how long the spores will remain viable, but growth of this species was obtained from dried soil from the original sample three years after collection. Germination is by a hypha which protrudes through a pore formed in the wall of the oospore (FIG. 23). This primary vegetative element almost immediately assumes the vacuolate condition typical of the mature mycelium.

***Monoblepharella Laruei* sp. nov.**

Mycelium well developed; hyphae sparingly branched, the branches usually arising at right angles to the main axes, delicate, 1.5-4 μ in diameter, the stouter basal portions up to 7 μ ; vacuolization reticulate or scalariform; hyphae with occasional swellings. Sporangia cylindrical or siliquiform, 28-82 μ long by 7-15 μ in diameter at the widest point, occurring at the tips of hyphae, or several often developed in basipetal succession, later sporangia sometimes geniculate. Zoospores emerging through a pore at the apex of the sporangium, ovoid or somewhat cylindrical, 7-9 μ long by



FIGS. 24-26, *Monoblepharella Laruei*; 47, *M. elongata*; 48 and 49, *M. Taylora*; 50, *M. mexicana*; 51, *M. Laruei*.

5–5.5 μ in diameter, with an anterior group of small refractive globules, posteriorly uniflagellate, the flagellum up to 27 μ in length. Oogonium terminal on a branch subtending the antheridium, or formed by a swelling of the hypha below the antheridium, after sympodial branching of the hypha appearing lateral, obpyriform, 11–20 μ long by 7–12 μ in diameter at the widest point, with rounded apex, and, if terminal, with narrowly cylindrical base tapering to 2–4 μ , the contents at maturity forming one or rarely up to three eggs bearing numerous large refractive globules. Antheridium always formed terminally on the hypha, later often epigynous, 8–19 μ long by 4–7 μ in diameter at the widest point. Antherozoids two to five, emerging through a pore at the apex of the antheridium, strongly amoeboid, ovoid when swimming, 4–5 μ long by 3–3.5 μ wide, with an anterior group of small refractive globules, posteriorly uniflagellate, the flagellum up to 19 μ in length. Zygote spherical or broadly ovoid, 10–13 μ long by 8–10 μ wide, posteriorly uniflagellate, free swimming, amoeboid during rest periods, the contents bearing numerous large refractive globules. Oospore formed free in the water, spherical, 9–13 μ in diameter, with a light brown, smooth wall up to 1 μ in thickness, contents bearing globules, germination not observed.

In soil, isolated on hemp seed bait. Soil collected by C. C. LaRue: stream bank mud, Cooper Landing, near Bluefields, Nicaragua, December 28, 1940 (type).

Mycelium amplum, hyphis ramosis, tenuibus 1.5–7 μ diametro, reticulate vel scalariforme vacuolatis, spatio interiecto tumoribus. Sporangia cylindrica vel siliquiformia 28–82 μ longa, 7–15 μ crassa, terminalia vel saepe plura basipetaliter producta; zoosporis ovoideis vel cylindricis 7–9 μ longis, 5–5.5 μ crassis, uno postico flagello usque ad 27 μ longo. Oogonium hypham antheridio subtenentem terminans aut e hypha sub antheridio tumescente formatum obpyriforme, apice rotundata 11–20 μ longum, 7–12 μ crassum, et si terminale basi anguste cylindrico 2–4 μ diametro; ovis singulis rare ad 3 cum globulis magnis refractivis. Antheridium semper hypham terminans, postea aliquando epigynum 8–19 μ longum, 4–7 μ crassum; antherozoides 2–5 valde amoeboides, ovoideis si natantibus, 4–5 μ longis, 3–3.5 μ crassis, uno postico flagello ad 19 μ longo. Ova inseminata sphaerica vel late ovoidea 10–13 μ longa, 8–10 μ crassa, natantia, dum requiescent amoeboides, magnis refractivis globulis inclusis. Oospora in aqua libere formata, sphaerica, 9–13 μ diametro, membrana pallide brunnea laevia ad 1 μ crassa, magnis refractivis globulis inclusis, germinatione ignota.

In humo per Cannabis semen illecebram culta. Humum legit C. D. LaRue e luto in rivi ripa, Cooper Landing, ad Bluefields, Nicaragua, December 28, 1940 (specimen typicum).

This fungus is dedicated to Dr. C. D. LaRue of the University of Michigan, who furnished a large number of the soil samples from which were obtained isolates of *Monoblepharella*, including the type isolates of *M. Laruei* and *M. elongata*.

The mycelium of *Monoblepharella Laruei* is typical for the genus. Some nodules are present although not in such great quantity as on hyphae of *M. elongata*. Sporangia are produced in great abundance when the mycelium is put into fresh water between temperatures of 21–31° C. They develop terminally on the hypha (FIG. 24) and in shape and size closely resemble those of *M. Taylori*. Secondary sporangia (FIGS. 25, 38) however, are formed in basipetal succession rather than in the sympodial manner characteristic of the other species. This type of sporangial formation has been found to occur in a few instances but not typically in cultures of *M. mexicana*. The swelling below the cross wall of the primary sporangium develops directly into a geniculate sporangium, which is then delimited basically from its attendant hypha by a cross wall. Sporangia may continue to form in this manner. Usually as each sporangium develops it comes to lie with its long axis in line with that of the hypha. The first formed sporangium now appears to be a lateral outgrowth from the mature secondary organ. Sporangial discharge and the zoospores (FIG. 26) are like those of other species.

Development of the sex organs proceeds in just the reverse order from that of *Monoblepharella elongata* and *M. Taylori*, starting with the formation of the antheridium, as in *M. mexicana*. The antheridium is formed by the swelling of a hyphal tip (FIG. 27) and the laying down of a cross wall (FIG. 28). The mature antheridium has the smallest average length (11 μ) of any of the isolates studied. The lateral outgrowth (FIG. 29) which soon forms beneath the antheridial cross wall may develop into a short branch, and produce a terminal oogonium (FIG. 34), or it may expand to form a subantheridial oogonium (FIGS. 30, 31). This method of oogonial development corresponds closely to that of the hypogynous antheridia of *M. Taylori* and *M. elongata*. When the oogonium is first cut off, the antheridium, although now epigynous, lies in a direct line with the long axis of the hypha. Frequently, however, the oogonium rather than the antheridium comes

to lie in this position, and the antheridium then appears lateral (FIGS. 32, 33). Occasionally a second female gametangium may be cut off immediately below the first (FIG. 35). By later sympodial branching of the hypha, the sex organs may assume a lateral position (FIG. 37).

The sequence of stages in the formation of the egg, involving the coalescence of oil droplets to form large globules, progresses as in other species. Rarely more than one egg may be formed in an oogonium (FIG. 36). Fully mature oogonia are obpyriform or geniculate, depending upon whether they are terminal or intercalary.

Sex organs have been developed abundantly in cultures kept at constant temperatures of between 26 and 32° C. If the culture is disturbed or removed from the oven, sporangial development will commence. Occasionally in such conditions sporangia will be formed basipetally beneath sex organs (FIG. 38).

Because of the small size of the antheridium, frequently there are only two antherozoids produced in each. The largest number observed in a single antheridium was five. The emergence of the antherozoids (FIGS. 36, 40, 41), their appearance when swimming (FIG. 39), fertilization (FIG. 40) and the emergence and the swarming of the zygote (FIGS. 41-45) are like these stages in other members of the genus. Large numbers of resting spores may be found in an old culture, but their germination has not as yet been observed.

Resemblances have been pointed out between *Monoblepharella Laruei* and *M. mexicana* and also between *M. elongata* and *M. Taylora*. Distinctive characteristics of the four species are emphasized by a comparison of drawings of typical pairs of sex organs. The organs of *Monoblepharella elongata* (FIG. 47) are much longer and comparatively narrower than the more rounded organs of *M. Taylora* (FIGS. 48, 49). In both of these fungi the oogonium is produced before its attendant antheridium, and the antheridium may be either terminal or hypogynous. In *Monoblepharella mexicana* (FIG. 50) and *M. Laruei* (FIG. 51) the oogonium develops after its attendant antheridium. In *M. mexicana* both organs are produced terminally on the hyphae, while in *M. Laruei* the oogonium is frequently epigynous.

SUMMARY

Two new species of *Monoblepharella* have been recovered from soil samples, *Monoblepharella elongata* from Mexico and California and *Monoblepharella Laruei* from Nicaragua. *Monoblepharella elongata* in the development and arrangement of its organs resembles *M. Taylori* Sparrow. It differs from Sparrow's species in the large number of nodules on the mycelium, the presence typically of lateral projections on the zoosporangia, the frequent formation of several eggs in the oogonia, and, most conspicuously, in the greater length and slenderness of its reproductive organs. *Monoblepharella Laruei* is the only known species of *Monoblepharella* in which epigynous antheridia may occur. This, the small size of the antheridia, and the formation of secondary zoosporangia almost exclusively in basipetal fashion are the chief characteristics which separate it from the closely related *M. mexicana* Shanor.

ACKNOWLEDGMENTS

The writer wishes to express her appreciation to Dr. C. D. LaRue for the use of the soils from which the new species were isolated, to Dr. Leland Shanor for the culture of *Monoblepharella mexicana*, and to Dr. F. K. Sparrow for the culture of *M. Taylori*. She is especially indebted to Dr. Sparrow for his helpful suggestions during the investigation and the preparation of this paper.

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EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida and are approximately $\times 758$ as here reproduced.

FIGS. 1-23, *Monoblepharella elongata*. 1, large zoosporangium with three lateral projections; 2, zoosporangium of average size; 3, zoospore; 4, zoosporangium showing late stage in emergence of zoospores; 5, young oogonium; 6, older oogonium and antheridial rudiment; 7, sex organs, with antheridium terminal; 8, sex organs, with antheridium hypogynous; 9, empty sex organs on a portion of mycelium bearing a nodule; 10, branch with two closely adjacent pairs of sex organs; antheridia hypogynous in both; 11, two pairs of sex organs, showing both terminal and hypogynous antheridia on the same branch; 12, large oogonium with eight eggs; 13, stage in emergence of antherozoids; 14, antherozoid; 15, antherozoid on tip of oogonium; 16-19, fertilization and stages in the emergence of the zygote; 20, typical motile zygote; 21, zygote with shape assumed during periods of amoeboid movement; 22, oospore; 23, germination of oospore.

FIGS. 24-46, *Monoblepharella Laruei*. 24, typical sporangium; 25, stage in discharge of zoospores from a terminal sporangium which is subtended by a basipetally formed secondary sporangium; 26, zoospore; 27-33, stages in development of sex organs, showing terminal development of antheridium, and subsequent development of oogonium so that the antheridium appears to be epigynous; 34, hyphal tip on which both the antheridium and the oogonium were formed terminally; 35, antheridium subtended by two oogonia produced in basipetal succession; 36, large oogonium with the two eggs; antheridium from which antherozoids are emerging; 37, portion of hypha showing sequence of sex organs; 38, hyphal tip showing basipetal development of four sporangia beneath an oogonium and epigynous antheridium; 39, antherozoid; 40, antherozoid about ready to fertilize egg; 41, 42, stages in emergence of zygotes; 43, typical motile zygote; 44, 45, shapes assumed by zygote during periods of amoeboid movement; 46, oospore.

FIGS. 47-51, typical sex organs of four species of *Monoblepharella*. 47, *M. elongata*; 48, 49, *M. Taylora*; 50, *M. mexicana*; 51, *M. Laruei*.

